# Report for 2001NM1661B: Genetic Techniques for the Verification and Monitoring of Dihaloethane Biodegradation in New Mexico Aquifers

- Water Resources Research Institute Reports:
  - Reiss, R. 2002. Genetic Techniques for the Verification and Monitoring of Dihaloethane Biodegradation in New Mexico Aquifers. NM Water Resources Research Institute, Technical Completion Report, #324. New Mexico State University, Las Cruces, NM. 63pp.

Report Follows:

**Research Project Objectives and Accomplishments**

This research project had two objectives: 1) determine the route of the gene (*dhlA*) in aquifers, and 2) determine which microbes may harbor the *dhlA* gene. The hypothesis was that the gene is present at very low levels until selection pressure is increased. Dihaloethanes can provide a rich carbon source for those microbes that harbor the genes to metabolize these compounds, so there is a strong selective advantage for those microbes that contain the *dhlA* gene in contaminated environments. Since the preliminary experiments involved isolating total DNA from water and sediment samples as a template for PCR, it is not known which microbes harbor this gene, or whether it is chromosomal or extrachromosomal. The hypothesis was that the gene will be found in a variety of naturally occurring microbes due to their ability to transfer genetic information laterally.

The first step for Objective 1 was to design a DNA isolation procedure that eliminates all inhibitors of DNA *Taq* polymerase. This enzyme amplifies DNA in the process of polymerase chain reaction (PCR). Modifications to the previously developed DNA isolation protocol include a microfiltration step. Next, the conditions to amplify the *dhlA* gene were established using DNA from *Xanthobacter autotrophicus* strain GJ10 as a positive control. A nested PCR strategy was designed in which two consecutive rounds of PCR are performed.

The method suggested to meet the second objective was to clone and sequence the 16SrRNA gene from all the microbes in the aquifer. A different approach is being developed to determine the microbial community of each aquifer. Rather than cloning and sequencing each individual 16S amplification produce, the 16S region is labeled with fluorescent primers, digested with restriction enzymes and electrophoresed on the Prism 310 Genetic Analyzer. The data are compared to the ribosomal database to determine the species.

**Application of Research Results**

Attempts to detect specific genes for dihaloethane biodegradation using polymerase chain reaction (PCR) in New Mexico aquifers proved unreliable. However, it was discovered that the enzyme activity was detectable in crude protein extracts made from groundwater samples. Direct enzyme assays for monitoring biodegradation potential has three major advantages over PCR. First, it is a direct measure of the activity, not just the potential. Just because a gene is present, it does not mean it is expressed. Second, it provides an accurate estimate even if more than one gene can be involved. Third, it is not prone to contamination because there is no amplification of product.

First-order biodegradation rate constants were calculated from rate constants determined from direct enzyme assays on crude protein extracts. This is a distinct improvement over current batch-reactor methods because it is a rapid method that does not require culturing so there is no loss of species from the consortium. These rate constants can be incorporated into existing models for natural attenuation.

The application of direct enzyme assays to monitoring of biodegradation does not require information regarding the microbial consortium of an aquifer. However, combining enzyme data with information regarding species diversity will advance the understanding of biodegradation. By comparing the enzyme data to the microbial consortium of numerous wells, a better understanding of the bacterial species responsible for biodegradation will result.